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Aflatoxin Reduction in Corn Through Field Application of Competitive Fungi†

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ABSTRACT

Soil in corn plots was inoculated with nonaflatoxigenic strains of *Aspergillus flavus* and *A. parasiticus* during crop years 1994 to 1997 to determine the effect of application of the nontoxigenic strains on preharvest aflatoxin contamination of corn. Corn plots in a separate part of the field were not inoculated and served as controls. Inoculation resulted in significant increases in the total *A. flavus/parasiticus* soil population in treated plots, and that population was dominated by the applied strain of *A. parasiticus* (NRRL 21369). In the years when weather conditions favored aflatoxin contamination (1996 and 1997), corn was predominately colonized by *A. flavus* as opposed to *A. parasiticus*. In 1996, colonization by wild-type *A. flavus* was significantly reduced in treated plots compared with control plots, but total *A. flavus/parasiticus* colonization was not different between the two groups. A change to a more aggressive strain of *A. flavus* (NRRL 21882) as part of the biocontrol inoculum in 1997 resulted in a significantly ($P < 0.001$) higher colonization of corn by the applied strain. Weather conditions did not favor aflatoxin contamination in 1994 and 1995. In 1996, the aflatoxin concentration in corn from treated plots averaged 24.0 ppb, a reduction of 87% compared with the aflatoxin in control plots that averaged 188.4 ppb. In 1997, aflatoxin was reduced by 66% in treated corn (29.8 ppb) compared with control corn (87.5 ppb). Together, the data indicated that although the applied strain of *A. parasiticus* dominated in the soil, the nonaflatoxigenic strains of *A. flavus* were more responsible for the observed reductions in aflatoxin contamination. Inclusion of a nonaflatoxigenic strain of *A. parasiticus* in a biological control formulation for aflatoxin contamination may not be as important for airborne crops, such as corn, as for soilborne crops, such as peanuts.

Aflatoxins are potent hepatotoxic, carcinogenic metabolites produced by the fungi *Aspergillus flavus* Link: Fr., *A. parasiticus* Speare, and *A. nomius* Kurtzman et al. *(2, 8, 18)*. These fungi have the capability of invading various agricultural commodities during maturation in the field or after harvest and contaminating them with aflatoxin. Commodities such as corn, peanuts, cottonseed, and various tree nuts are particularly susceptible to preharvest aflatoxin contamination when environmental conditions during crop maturation are characterized by high temperatures and moisture stress and when insect injury is prevalent *(4, 7, 12, 20)*. Because of the toxicity and carcinogenicity of aflatoxins, contaminated commodities destined for human or animal consumption pose a significant health hazard and are, therefore, closely monitored and regulated *(24)*. Apart from its effect on health, aflatoxin contamination also impacts the agricultural economy through the loss of produce and the time and costs involved in monitoring and decontamination efforts *(22)*.

Major research efforts are underway to develop and implement various aflatoxin control strategies. These efforts include research in the areas of breeding for crop resistance, genetic engineering for crop resistance, regulation of aflatoxin biosynthesis, and biological control. Biological control efforts have centered on the inoculation of crop soils with competitive, nonaflatoxigenic strains of *A. flavus* and *A. parasiticus*. Application of competitive, nonaflatoxigenic strains of *A. parasiticus* reduced aflatoxin contamination of peanuts over a 3-year period *(10)*, and a patent was granted for the use of nonaflatoxigenic strains of *A. parasiticus* for controlling aflatoxin contamination *(3)*. A 2-year study in which different rates of inoculum of nonaflatoxigenic strains of *A. flavus* and *A. parasiticus* in combination were applied to peanut soil showed reductions in aflatoxin contamination of peanuts ranging from 74.3 to 99.9% *(11)*. Reduction in aflatoxin contamination of cottonseed has also been achieved by application of a nonaflatoxigenic strain of *A. flavus* to soil around developing cotton plants *(6)*, and a patent was issued for the use of nonaflatoxigenic *A. flavus* for preventing aflatoxin contamination *(5)*. Brown et al. *(1)* found that aflatoxin contamination of corn was reduced when developing corn kernels were directly coinoculated with toxigenic and nonaflatoxigenic strains of *A. flavus* as opposed to inoculation with the toxigenic strain alone. Aflatoxin was also reduced when kernels were inoculated with the nonaflatoxigenic strain 24 h prior to inoculation with the aflatoxin-producing strain. However, no reports have been published concerning the effect of soil inoculation

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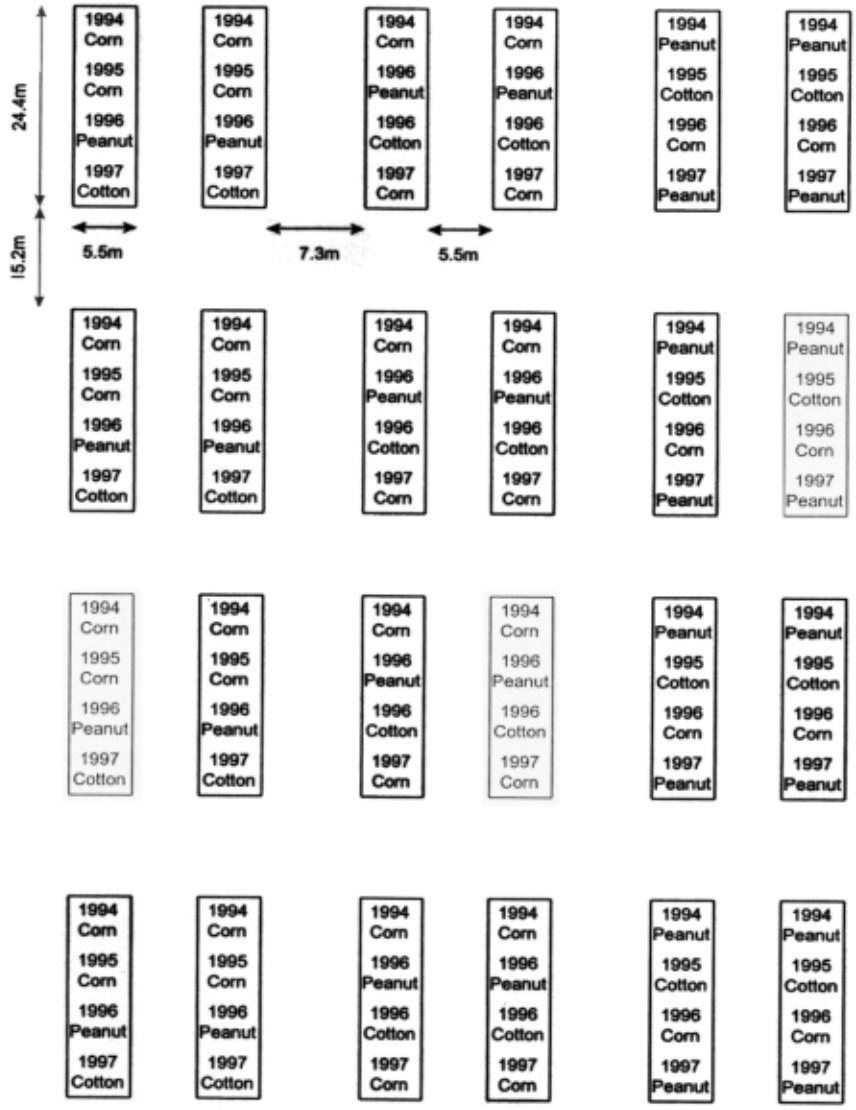

FIGURE 1. *Layout of plots treated with competitive, nonaflatoxigenic strains of* A. flavus *and* A. parasiticus *showing the rotation of corn, peanuts, and cotton among plots for crop years 1994 to 1997. An equivalent layout of nontreated control plots was established about 1 km away in the same noncultivated field.*

with nonaflatoxigenic strains on preharvest aflatoxin contamination of corn. The purpose of this paper is to report the reduction in aflatoxin contamination of corn that was achieved through soil inoculation with nonaflatoxigenic strains of *A. flavus* and *A. parasiticus*.

MATERIALS AND METHODS

Fungal strains and inoculum preparation. Strains of *A. flavus* and *A. parasiticus* used as competitive fungi in this study included a naturally occurring isolate of *A. flavus* (NRRL 21882), an orange-brown color mutant *A. flavus* (NRRL 21368) produced by UV irradiation of NRRL 21882, and an orange-brown color mutant of *A. parasiticus* (NRRL 21369), all of which lacked the ability to produce aflatoxins, cyclopiazonic acid, and known biosynthetic precursors of aflatoxin *(11)*. Cultures were maintained on Czapek's agar (Cz) slants at 5°C. Soil inoculum was prepared by culturing each strain separately on long-grain rice as previously described *(11)*.

Field plots and inoculation. Twenty-four (each 5.5 by 24.4 m) plots were delineated in a portion of an otherwise noncultivated field for treatment with the competitive fungi. Figure 1 illustrates the arrangement and spacing of plots and crops planted

within each plot. In 1994, 16 of the 24 plots each were planted with six rows of corn while the other 8 plots were planted with six rows of peanuts. In 1995, eight of the 1994 corn plots were replanted with corn, eight were planted with peanuts, and the 1994 peanut plots were planted with cotton. In subsequent years, corn plots were rotated with peanuts followed by cotton, cotton plots were rotated with corn followed by peanuts, and peanut plots were rotated with cotton followed by corn. Equivalent nontreated control plots were established in a portion of the field about 1 km removed from the treated plots. A noncultivated field with separation between the treated and control plots was selected in order to limit any influence of soil microflora associated with treated plots upon control plots and vice versa as well as any influence of fungi associated with a developing crop upon treated or control plots. Cotty *(6)* observed that when a nonaflatoxigenic strain of *A. flavus* was applied to treated plots interspersed with untreated plots in the same cotton field, the applied strain was isolated from as many as 25% of infected seed in the untreated plots and probably reduced aflatoxin contamination in the untreated plots.

Treated plots were inoculated mechanically each year with fungal-infested rice using a Gandy applicator pulled behind a tractor. Inoculum was banded over the row when corn plants were approximately 30 to 60 cm high. Planting and inoculation dates, respectively, for corn plots for years 1994 to 1997 were as follows: 1994, 17 May and 22 June; 1995, 15 March and 27 April; 1996, 5 March and 8 May; 1997, 13 March and 23 May. In 1994 to 1996, treated plots were inoculated at a rate of 225 kg/ha with an equal mixture of rice colonized by *A. flavus* (NRRL 21368) and *A. parasiticus* (NRRL 21369) color mutants. In 1997, treated plots were inoculated at the reduced rate of 22.5 kg/ha with an equal mixture of *A. flavus* (NRRL 21882) and *A. parasiticus* (NRRL 21369).

Harvesting and preparation of samples. Corn plots were harvested on 30 September 1994, 15 August 1995, 18 September 1996, and 26 August 1997, with a commercial combine. After each plot was harvested, the corn was transferred to burlap bags. Yield varied among plots and years but typically averaged around 80 kg per plot. Bags of corn from each plot were combined and riffle divided to produce a sample of about 20 kg to grind for aflatoxin analysis. Corn was ground in a Romer subsampling mill (Romer Labs, Union, Mo.), and subsamples averaging 1,275 g were analyzed for aflatoxin. Corn was further riffle divided to produce a sample of approximately 500 g to be used for the determination of fungal colonization percentages.

Soil microfloral analyses. Samples of soil from each plot were taken at the time of planting and immediately prior to harvest to monitor populations of wild-type *A. flavus* and *A. parasiticus* as well as the introduced competitive strains. Ten samples of soil were removed with a sterile trowel from the top 6 cm of soil within 15 cm of the planted kernels or corn stems at random locations within each plot. Samples within a plot were pooled, mixed, and stored in a paper bag. Soil was screened through a no. 20 standard testing sieve (0.833-mm opening), and 30 g was blended for 1 min at low speed with 300 ml of a 0.2% water agar solution that was cooled to 5°C *(13)*. Dilution platings were made on plates containing a dichloran-rose bengal medium *(17)* modified with 3% NaCl. Plates were incubated at 30°C for 3 to 4 days. Color mutants were readily distinguishable from wild-type strains of *A. flavus* and *A. parasiticus*, but the wild-type strains of *A. flavus* and *A. parasiticus* were not enumerated separately. The competitive *A. flavus* strain, NRRL 21882, that was applied only in 1997 was not differentiated from naturally occurring wild-type strains.

Fungal colonization of corn. In each year except 1995, sound, intact corn kernels were plated to determine the percentage of kernels that were colonized by the introduced competitive strains versus naturally occurring wild-type strains of *A. flavus* and *A. parasiticus*. In 1994, 1996, and 1997, 200 kernels from each plot were surface sterilized with a 2% solution of sodium hypochlorite and placed in petri dishes (five kernels per plate) containing Cz with antibiotics (30 μg/ml streptomycin and 1.5 μg/ml chlortetracycline) and incubated for 5 to 7 days at 30°C. Grain samples harvested in 1996 were also examined for fungi at NCAUR, Peoria, Ill. Fifty kernels per plot were surface sterilized with a 0.5% hypochlorite solution, plated on 3% malt extract agar, and incubated for 5 to 7 days at 25°C. A second plating of 50 kernels per plot followed surface sterilization with a 2% hypochlorite solution. Wild-type strains of *A. flavus* and *A. parasiticus* were not differentiated, but color mutants NRRL 21368 and 21369, used as competitive fungi, were readily distinguishable from wild types and from each other.

Because of reproducible results from the three plating experiments in 1996, only one plating series was done in 1997. It included 200 kernels per plot that were surface sterilized with a 2% hypochlorite solution. To differentiate between the applied wild-type strain of *A. flavus* (NRRL 21882) and native strains of *A. flavus* that are visually indistinguishable, up to 20 *A. flavus* colonies per plot were subcultured in 1 ml of a liquid medium containing glucose, soytone, yeast extract, and sucrose in 1-dram vials *(15)*. After incubation at 30°C for 7 days, cultures were extracted with chloroform and analyzed by thin-layer chromatography (TLC) on silica gel 60 F-254 plates with a solvent system of chloroform-acetone (93/7, vol/vol). A match of the metabolite pattern with that of an extract of NRRL 21882, particularly the absence of aflatoxins and cyclopiazonic acid, was used to identify a colony as the applied competitive strain.

Aflatoxin analyses. Ground corn subsamples were extracted in a Waring gallon-sized blender for 3 min with methanol-water (80/20, vol/vol; 2 ml/g) and analyzed for aflatoxin by the high-performance liquid chromatography (HPLC) method of Dorner and Cole *(9)* with certain modifications. The HPLC system consisted of a Waters Nova-PAK C_{18} column (3.9 by 150 mm) with a mobile phase of water-methanol-butanol (700/355/12, vol/vol/vol). Instead of using postcolumn iodination to enhance fluorescence of aflatoxins B_1 and G_1, postcolumn derivatization was achieved with a photochemical reactor *(16)* placed between the column and a Shimadzu model RF551 fluorescence detector with excitation and emission wavelengths of 365 and 440 nm, respectively. Injection solvent consisted of methanol-water (62/38, vol/vol) with 0.1% acetic acid. Aflatoxin standards were prepared from crystals according to AOAC method 971.22 *(21)*.

Statistical analyses. Soil populations and aflatoxin concentrations were log transformed to normalize distributions. Corn colonization percentages were transformed to the arc sine of the square root. Analyses of variance and *t* tests were run with the general linear models procedure of SAS including a Tukey means separations test to determine differences among means *(23)*.

RESULTS

Soil microflora. Soil populations of wild-type and nonaflatoxigenic, competitive strains of *A. flavus* and *A. parasiticus* are presented in Table 1. Populations in control plots ranged from 65 CFU/g at planting in 1996 to 478 CFU/g at harvest in 1995. Populations decreased significantly between planting and harvest in 1994 but increased

TABLE 1. *Soil populations and distribution of* Aspergillus flavus *and* A. parasiticus *in control plots and plots treated with nonaflatoxigenic, competitive strains of* A. flavus *and* A. parasiticus

Sampling time	Control CFU/g[a]	Treated CFU/g[b]	% colonies in treated plots belonging to		
			Wild type[c]	*A. flavus* CM[d]	*A. parasiticus* CM[e]
Planting, 1994	311 AB	163 C	100	0	0
Harvest, 1994	83 C	9,537 B	1	32	67
Planting, 1995[f]	81 BC	3,003 BC	3	17	80
Harvest, 1995	478 A	41,190 A		15	84
Planting, 1996[f]	65 BC	4,878 BC	2	11	87
Harvest, 1996	231 ABC	1,770 C	11	18	71
Planting, 1997[f]	302 ABC	5,083 BC	4	1	95
Harvest, 1997	125 BC	2,439 C	25[g]	4	71

[a] Values are the mean of eight plots and include all strains of *A. flavus* and *A. parasiticus*. Values in a column followed by the same letter are not significantly different (two-way ANOVA, Tukey least-squares means test, $P < 0.05$).

[b] Values are the mean of eight plots and include all strains of *A. flavus* and *A. parasiticus*. Values in a column followed by the same letter are not significantly different (two-way ANOVA, Tukey least-squares means test, $P < 0.05$).

[c] Includes wild-type strains of *A. flavus* and *A. parasiticus*.

[d] Competitive color mutant strain of *A. flavus*, NRRL 21368.

[e] Competitive color mutant strain of *A. parasiticus*, NRRL 21369.

[f] Populations in treated plots include carryover from the previous year.

[g] Includes nonaflatoxigenic *A. flavus*, NRRL 21882, applied as a biocompetitive agent in 1997.

significantly in 1995. In 1996 and 1997 populations did not change significantly between planting and harvest samplings. Individual *t* tests for control versus treated means for each sampling showed that except for the initial sampling prior to any inoculation, treated plots always had significantly ($P < 0.05$) higher total *A. flavus/parasiticus* populations than control plots. Treated plots had significant increases between planting and harvest during 1994 and 1995 resulting from inoculation with the competitive fungi, but no differences were seen between planting and harvest populations in 1996 and 1997.

In treated plots, the *A. flavus* and *A. parasiticus* soil populations were dominated by the applied color mutant of *A. parasiticus* (NRRL 21369) that made up from 67 to 95% of those populations. The *A. flavus* color mutant (NRRL 21368) comprised only 11 to 32% of the total *A. flavus/parasiticus* population during years when it was part of the inoculum (1994 to 1996). This apparent lack of dominance by the *A. flavus* color mutant compared with the *A. parasiticus* color mutant prompted a change in 1997 to the nontoxigenic wild-type, NRRL 21882, from which the *A. flavus* color mutant was derived. An increase in the wild-type population at harvest in 1997 was reflective of the presence of that strain. However, the change did not result in any reduction in soil dominance by the *A. parasiticus* color mutant that again comprised 71% of the total *A. flavus/parasiticus* population at harvest, 1997.

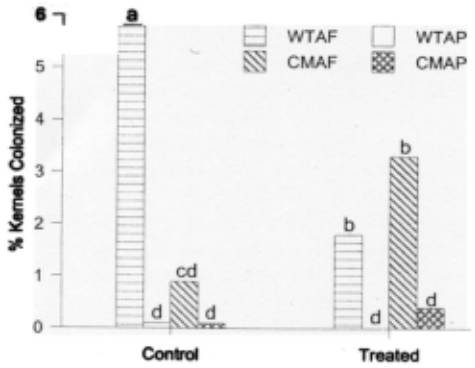

FIGURE 2. *Percentage of corn kernels colonized by wild-type* A. flavus *(WTAF), wild-type* A. parasiticus *(WTAP),* A. flavus *color mutant NRRL 21368 (CMAF), and* A. parasiticus *color mutant NRRL 21369 (CMAP) in control and treated plots in 1996. Bars with the same letter are not significantly different (two-way ANOVA, Tukey least-squares means test,* $P < 0.05$*).*

Fungal colonization of corn. Essentially no colonization of corn by *A. flavus/parasiticus* occurred in 1994. Only 2 kernels out of 3,200 plated yielded colonies of *A. flavus*; however, a high percentage (70 to 80%) of kernels was colonized by *Fusarium* spp. This was likely the result of a very wet and cool growing season. The percentage of kernels colonized by wild-type and color mutant strains of *A. flavus* and *A. parasiticus* in 1996 is presented in Figure 2. Corn was primarily colonized by strains of *A. flavus* as opposed to *A. parasiticus*. In control plots, 5.8% of kernels were colonized by wild-type *A. flavus*, whereas only 0.1% were colonized by wild-type *A. parasiticus*. The competitive color mutants of *A. flavus* (NRRL 21368) and *A. parasiticus* (NRRL 21369) also colonized 0.9 and 0.1% of the kernels, respectively, even though the treated and control plots were separated by a distance of 1 km. Similarly in the treated plots, corn was predominately colonized by *A. flavus* as opposed to *A. parasiticus* with 1.8 and 3.3% of kernels colonized by wild-type and color mutant *A. flavus*, respectively. Only 0.4% of kernels in treated plots were colonized by *A. parasiticus*, and that being the color mutant.

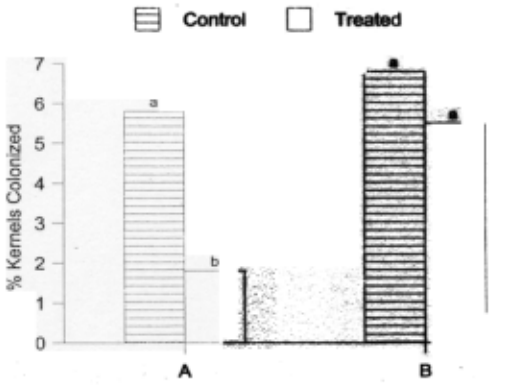

FIGURE 3. *Colonization of corn kernels by wild types of* A. flavus *and* A. parasiticus *(A) and by all strains of* A. flavus *and* A. parasiticus *(B) in control and color mutant-treated plots in 1996. Bars with the same letter are not significantly different (two-way ANOVA, Tukey least-squares means test,* $P < 0.001$*).*

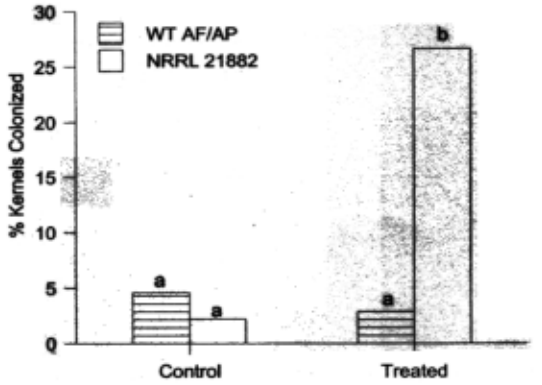

FIGURE 4. *Colonization of corn kernels by wild-type* A. flavus/parasiticus *(WT AF/AP) and by* A. flavus, *NRRL 21882, used as a competitive fungus in 1997. Bars with the same letter are not significantly different (two-way ANOVA, Tukey least-squares means test,* $P < 0.001$*).*

The comparison of the percent kernels colonized by wild-type strains of *A. flavus* and *A. parasiticus* and by all strains of *A. flavus* and *A. parasiticus* in control and treated plots from 1996 is shown in Figure 3. The two-way analysis of variance (ANOVA) showed no significant differences among the three plating experiments; therefore, data are presented from all three experiments combined. A highly significant ($P < 0.001$) reduction in colonization by wild-type *A. flavus* was found in treated plots (1.8%) compared with control plots (5.8%) (Fig. 3A). However, no difference was found between treated (5.5%) and control plots (6.8%) for total colonization by both wild-type *A. flavus/parasiticus* and the applied color mutants (Fig. 3B). This was in spite of the fact that total *A. flavus/parasiticus* soil populations were significantly higher in treated plots (Table 1). These data demonstrate that application of the competitive color mutants did not alter overall colonization of corn by *A. flavus/parasiticus,* but it did result in a significantly reduced colonization by wild-type, toxigenic strains.

The colonization of corn in 1997 by wild-type *A. flavus/parasiticus* and the applied nontoxigenic *A. flavus* (NRRL 21882) is illustrated in Figure 4. Two-way ANOVA with means separated by Tukey's least-squares means test showed a significantly ($P < 0.001$) larger percentage (26.7%) of kernels colonized by the applied strain of *A. flavus* in treated plots. The difference between colonization by wild-type *A. flavus* in control (4.6%) and treated (2.9%) plots was not significant. Although control plots were well removed from treated plots, 2.2% of kernels in control plots were colonized by the competitive strain applied to treated plots. Only 2 kernels out of 1,600 plated from treated plots were colonized by the *A. parasiticus* color mutant, even though that fungus again dominated in the soil (Table 1). The *A. flavus* color mutant, which was not applied to soil in 1997 but still present from prior applications, colonized only one kernel.

Aflatoxin contamination. In 1994 and 1995 only traces of aflatoxin were found due to relatively cool and wet environmental conditions. The mean aflatoxin concentrations in corn from control and treated plots in 1996 and 1997 are shown in Figure 5. Significant ($P < 0.01$) reductions in aflatoxin were achieved in each year. The mean aflatoxin concentration of 24.0 ppb from treated plots in 1996 represented a reduction of 87% compared with the control plots average of 188.4 ppb. A 66% reduction was seen in 1997 with control corn averaging 87.5 ppb compared with 29.8 ppb in corn from treated plots. Of total aflatoxin in 1996, 89% was aflatoxin $B_1 + B_2$ and 11% was aflatoxin $G_1 + G_2$ in both treated and control plots. In 1997, 96% of the total aflatoxin in treated plots was $B_1 + B_2$ while 89% of the total in control plots was $B_1 + B_2$.

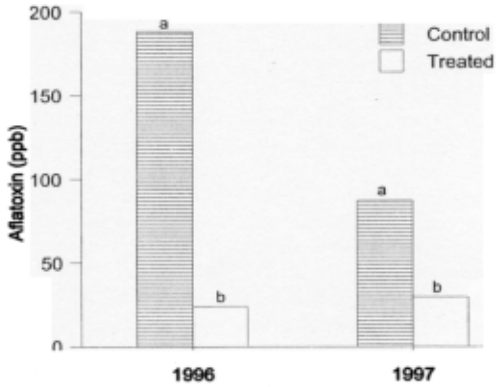

FIGURE 5. *Mean aflatoxin concentrations (ppb) in corn from control plots and plots treated with competitive strains of* A. flavus *and* A. parasiticus *in 1996 and 1997. Bars in the same year with different letters are significantly different (*t *test,* $P < 0.05$*).*

DISCUSSION

Applications of nonaflatoxigenic strains of *A. flavus* and *A. parasiticus* greatly altered the overall populations of those species in soil. Although wide fluctuations were seen in the *A. flavus/parasiticus* soil populations in plots that were treated with the nontoxigenic strains (Table 1), the percentage of total *A. flavus/parasiticus* that were wild strains was usually <5%. The *A. parasiticus* color mutant usually accounted for >70% of the total *A. flavus/parasiticus* population. Although the strains used in this study were specific color mutants of *A. flavus* and *A. parasiticus* (with the exception of NRRL 21882 in 1997), the data support earlier studies showing the tendency of *A. parasiticus* to dominate in the soil environment *(8)*.

The fungal colonization data show that even with the predominance of the *A. parasiticus* color mutant in the soil, corn was predominately invaded by *A. flavus* in 1996 and 1997. At harvest in 1996, the *A. parasiticus* color mutant made up 71% of the *A. flavus/parasiticus* soil population, but only 7% of kernels colonized by *A. flavus/parasiticus* were colonized by that strain while 93% of colonized kernels were colonized by *A. flavus*. At harvest in 1997 when the *A. parasiticus* color mutant again made up 71% of the *A. flavus/parasiticus* soil population, essentially all colonization of corn was by *A. flavus*. Numerous earlier studies have shown that among species of *Aspergillus* in section *Flavi*, *A. flavus* is usually the predominant colonizer of crops, even when soil populations of *A. parasiticus* have been as high or higher. In a 3-year study that monitored soil populations of *A. flavus* and *A. parasiticus*, Horn et al. *(14)* showed that while the two species were present in soil in similar proportions, corn was infected only by *A. flavus*. They suggested that *A. flavus* may be the more aggressive species on corn and may out compete *A. parasiticus* as a colonist of corn under field conditions. Horn et al. *(13)* reported that despite higher soil populations of a brown conidial mutant of *A. parasiticus*, *A. flavus* was usually dominant in peanut seeds. Lillehoj et al. *(19)* found *A. parasiticus* only in soil and soil insects in corn plots, whereas *A. flavus* was associated with soil and plant insects. However, *A. parasiticus* was not excluded as a colonist of corn in this present study, and the presence of the G aflatoxins (produced only by *A. parasiticus*) in both 1996 and 1997 is indicative of at least some invasion by that species.

Many factors are involved in the process of infection of corn kernels by *A. flavus/parasiticus*, but weather conditions are probably the most important factor determining whether or not aflatoxin contamination will develop *(20)*. In 1994, essentially no kernels were colonized by *A. flavus/parasiticus* even though the application of *A. flavus/parasiticus* color mutants resulted in relatively high soil populations by the time of harvest. The 1994 growing season was characterized by abundant rainfall and unusually cool temperatures for southwestern Georgia. This, instead, resulted in heavy colonization of corn by *Fusarium* species and practically no colonization by *A. flavus/parasiticus*, in spite of the high *A. flavus/parasiticus* soil populations in treated plots.

The soil population data along with the kernel colonization data from this and an earlier study with peanuts *(11)* indicate that the biological control of aflatoxin contamination achieved in this study is not associated with a reduction in the propagules of toxigenic strains in treated soil. In fact, populations of wild-type strains of *A. flavus* and *A. parasiticus* usually were not different between treated and control soils even though the population of applied strains was much higher in treated soil. The competitiveness of the applied strains in the process of colonizing and becoming established in crops is a vital aspect in the success of this strategy. Although it was not dominant in the soil as far as total propagules were concerned, *A. flavus* NRRL 21882 was clearly more aggressive as a colonist of corn kernels. *A. parasiticus*, NRRL 21369, was an infrequent colonist of corn even though it maintained the highest propagule levels in the soil.

It appears that application of nontoxigenic *A. parasiticus* to soil may not be important in controlling aflatoxin in corn, but the strain of *A. flavus* that is used as a biocompetitive agent is very important. The color mutant strain of *A. flavus* was not as aggressive as the nontoxigenic wild type used in this study based on colonization percentages (Figs. 1 and 3). In a soilborne crop such as peanuts, it may be important to use a combination of nontoxigenic strains of *A. flavus* and *A. parasiticus* for biological control *(13)*. Although *A. flavus* dominates over *A. parasiticus* in peanut seeds, *A. parasiticus* is still an important contributor to overall aflatoxin contamination in peanuts *(8)*. However, because aflatoxin contamination is usually the result of infection only by *A. flavus* in the aerial crops, the use of nontoxigenic *A. parasiticus* as part of a biocontrol inoculum for those crops may not be necessary unless those crops are part of a crop rotation with peanuts.

The reduction in preharvest aflatoxin contamination of corn seen in this study does not indicate that this biocontrol strategy offers a solution to the problem of aflatoxin contamination in corn. However, when coupled with other research strategies being formulated and tested, it may provide a portion of the protection needed to impact both the health and economic consequences of aflatoxin contamination.

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